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PROTEASES OF STORED PRODUCT INSECTS AND THEIR
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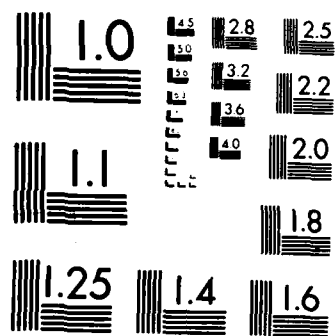
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20. ABSTRACT (Continue on reverse side if necessary and identify by block number) The research covered by the first Interim Report was devoted to detection, isolation and characterization of digestive proteinases of the rust red flour beetle (<u>Tribolium castaneum</u>), the mealworm (<u>Tenebrio molitor</u>), and the locust (<u>Locusta migratoria</u>), and to their interaction with synthetic and naturally-occurring proteinaceous protease inhibitors. Larval midguts of <u>Tribolium</u> <u>castaneum</u> larvae exhibited pronounced trypsin- and cont...		

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INTERIM REPORT

PROTEASES OF STORED PRODUCT INSECTS AND THEIR INHIBITION BY
SPECIFIC PROTEASE INHIBITORS FROM SOYBEANS AND WHEAT GRAIN

PRINCIPLE INVESTIGATORS:

YEHUDITH BIRK
DEPARTMENT OF BIOCHEMISTRY AND HUMAN NUTRITION

SHALOM W. APPLEBAUM
DEPARTMENT OF ENTOMOLOGY

FACULTY OF AGRICULTURE
THE HEBREW UNIVERSITY OF JERUSALEM
P.O. BOX 12
REHOVOT 76100, ISRAEL

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PREFACE

It is assumed that the physiological function of plant proteinase inhibitors in general and of seeds in particular is to protect the plant against attack by insects. The investigation of the digestive proteases of several model insects is a pre-requisite for understanding the complex relationship that the insects have evolved with the plant. The information on insect proteolytic enzymes in comparison to the corresponding proteases from higher organisms is essential for studying the selective interactions of the naturally-occurring protease inhibitors with the insect proteases.

The research covered by the first Interim Report primarily was devoted to detection, isolation and characterization of digestive proteinases of the rust red flour beetle (Tribolium castaneum), the mealworm (Tenebrio molitor), and the locust (Locusta migratoria). Recently, the latter has been posing a serious threat to valuable crops in different parts of the world.

EXPERIMENTAL RESULTS

(1) Tenebrio molitor proteases

Trypsin, chymotrypsin and carboxypeptidase B are major constituents of the digestive enzymes in Tenebrio molitor larvae and adults. In Tenebrio molitor, both the pupal and adult midgut epithelia are reformed during metamorphosis by the proliferation of cells from the posterior end of the foregut. It was particularly intriguing to clarify whether the composition and structure of proteolytic enzymes in the adult remain the same as in the larva.

The following results relate to the isolation and characterization of trypsin and chymotrypsin from the alimentary system of Tenebrio molitor adults. Preliminary experiments with different parts of the guts indicated that most of the Tenebrio trypsin and chymotrypsin is concentrated in the midgut. The isolation of Tenebrio adult trypsin and chymotrypsin was achieved by column-chromatography on the anion exchanger DEAE-cellulose followed by affinity chromatography on p-aminobenzamidinsepharose and phenylbutylamine-sepharose, respectively.

Tenebrio trypsin and Tenebrio chymotrypsin showed single homogenous bands upon electrophoresis on cellulose acetate membranes at pH 7.3 and on polyacrylamide gels at pH 4.5.

The molecular weight of Tenebrio trypsin was determined as 16700 by sodium dodecyl sulfate polyacrylamide gel electrophoresis and as 16500 by exclusion chromatography on a Sedphadex-G-50 column. The molecular weight of Tenebrio chymotrypsin was estimated from amino acid composition as 16400.

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The kinetic properties of Tenebrio trypsin and chymotrypsin were determined with the respective substrates tosyl-L-arginine methyl ester and acetyl-L-tyrosine ethyl ester and were compared to bovine trypsin and chymotrypsin. Both enzymes were fully inhibited by specific, synthetic inhibitors: Tenebrio trypsin - by N- α -tosyl-L-lysine chloromethyl ketone (TLCK) and by p-aminobenzamidine and Tenebrio chymotrypsin - by N- α -tosyl-phenylalanine chloromethyl ketone (TPCK) and by phenylbutylamine. They were also fully inhibited by the proteinaceous, doubleheaded trypsin- and chymotrypsin-inhibitors from legume seeds, such as the Bowman-Birk Inhibitor (BBI) from soybeans and by CI, the inhibitor from chick peas.

The amino acid analyses given in Table 1 clearly demonstrate that trypsin and chymotrypsin from Tenebrio adults differ in amino acid composition from the respective enzymes of Tenebrio larvae and from the bovine and carp enzymes. The complete lack of disulfide bonds in the Tenebrio proteases as compared to the six S-S bonds in bovine trypsin, suggests a significant difference in conformation. The relatively low molecular weight of Tenebrio trypsin and chymotrypsin is remarkable. No zymogens of Tenebrio trypsin or chymotrypsin have been found thus far.

(2) Tribolium castaneum proteases

Insect cultures of Tribolium castaneum larvae have been maintained at 32°C on commercial white wheat flour supplemented with 5% brewers yeast. Larval midguts of last instar larvae have been used for preparation of aqueous midgut enzyme extracts. The latter exhibited pronounced trypsin and chymotrypsin-like activities when assayed on the substrates tosyl-L-arginine methyl ester and benzoyl-arginyl-p-nitro-anilide, which are specific for trypsin and on the substrates acetyl-L-tyrosine ethyl ester and acetyl-tyrosine p-nitro anilide - for chymotrypsin. These activities have been fully inhibited by the above-mentioned chloromethyl ketones TLCK and TPCK which are specific active site titrants of trypsin and chymotrypsin, respectively. Moreover, they were also fully inhibited by the trypsin - chymotrypsin inhibitors BBI from soybeans and CI from chickpeas. Attempts are now in progress to separate and isolate these trypsin-and chymotrypsin-like enzymes.

(3) Locust proteinases

Preliminary experiments on the digestive tract of the locust have clearly indicated that the caecum is the optimal source for digestive proteases.

TABLE 1

COMPARISON OF AMINO ACID COMPOSITION OF TRYPSINS
AND CHYMOTRYPSINS FROM DIFFERENT SOURCES

AMINO ACID	TRYPSIN				CHYMOTRYPSIN			
	<i>Tenebrio molitor</i>		BOVINE	CARP	<i>Tenebrio molitor</i>		BOVINE	CARP
	larvae	adults			larvae	adults		
	K		3	3			T	3
LYSINE	3	4	14	10	6	16	14	9
HISTIDINE	3	3	3	6	3	2	2	6
ARGININE	6	4	2	7	7	8	4	9
ASPARTIC ACID	16	23	22	27	20	15	23	23
THREONINE	13	10	10	10	12	10	23	18
SERINE	33	14	33	22	14	13	28	24
GLUTAMIC ACID	13	18	14	26	22	13	15	14
PROLINE	8	8	9	9	10	11	9	12
GLYCINE	30	19	25	28	21	15	23	22
ALANINE	16	13	14	14	19	11	22	21
HALF CYSTINE	4	0	12	12	8	1	10	8
VALINE	19	13	17	18	15	11	23	22
METHIONINE	1	0	2	5	2	1	2	2
ISOLEUCINE	12	9	15	10	8	8	10	13
LEUCINE	13	16	14	12	12	11	19	19
TYROSINE	8	4	10	8	5	3	4	6
PHENYLALANINE	1	4	3	4	7	4	6	4
TRYPTOPHAN	0	8	4	8	*	*	8	8
TOTAL	199	171	223	236	191	153	245	240
MOLECULAR WEIGHT	19 768	18 382	23 800	25 567	19 622	16 380	25 600	25 570

* Not determined

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Trypsin and chymotrypsin have been separated from aqueous extracts of the caecae by means of ion-exchange-chromatography on a DEAE-cellulose column. The trypsin appeared in the anionic fraction and the chymotrypsin in the cationic fraction. Further purification by means of affinity chromatography as well as characterization of these enzymes are now in progress.

(4) Proteinase inhibitors from seeds

In order to study the interaction of the naturally-occurring proteinase inhibitors with the insect proteinases, we have attempted the preparation of native and modified trypsin-chymotrypsin inhibitors from soybeans (BBI) and from chickpeas (CI). In addition, a specific Tribolium proteinase inhibitor from soybeans was separated.

SIGNIFICANT FINDINGS

- A. The detection of trypsin- and chymotrypsin-like enzymes in the midgut of Tribolium castaneum larvae suggests that this insect may be susceptible to inhibition by naturally-occurring trypsin- and chymotrypsin-inhibitors.
- B. The findings that trypsin and chymotrypsin from the digestive tract of Tenebrio adults differ in amino acid composition from the respective larval enzymes indicates that the composition and consequently, the structure of the proteolytic enzymes in the adult do not remain the same as in the larva after metamorphosis. (ISRAEL) ←

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